# A Convenient Synthesis of (22*S*)-22-Hydroxycampesterol and Some Related Steroids

**Suguru Takatsuto,*[a] Tsuyoshi Watanabe,[b] Chiharu Gotoh,[a] Hiroki Kuriyama,[b] Takahiro Noguchi[b] and Shozo Fujioka[c]**

[a]*Department of Chemistry, Joetsu University of Education, Joetsu-shi, Niigata 943, Japan*
[b]*Tama Biochemical Co. Ltd., 2-7-1 Nishishinjuku, Shinjuku-ku, Tokyo 163, Japan*
[c]*The Institute of Physical and Chemical Research (RIKEN), Wako-shi, Saitama 351-01, Japan*

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As possible candidates for intermediates in brassinolide biosynthesis, (22*S*)-22-hydroxycampesterol **1** and its related new steroids **5**–**7** are conveniently synthesized by employing a Grignard reaction of a known steroidal 22-aldehyde **8** with 2,3-dimethylbutylmagnesium bromide as a key reaction.

We have previously reported a synthesis of (22*S*)-22-hydroxycampesterol **1**, 6-deoxocathasterone **2**, 6α-hydroxycathasterone **3** and cathasterone **4** as possible candidates for intermediates in brassinolide biosynthesis.[2] During the course of our metabolic study of deuterio-labelled campesterol in the cultured cells of *Catharanthus roseus*, we have very recently found that some 22-hydroxylated steroids occur both as natural products and as metabolites of the labelled campesterol.[3] Furthermore, we have recently demonstrated that *Arabidopsis* dwarf mutant *dwf4* is brassinosteroid-deficient and that light-grown dwarf seedlings grown on **1**–**4** and all the downstream compounds belonging to the early and late C-6 oxidation pathways[4a] of the brassinolide biosynthesis rescued the *dwf4* phenotype, while the known precursors without a 22*S*-hydroxy group failed to cause an elongation response.[5] In addition, using another *Arabidopis* dwarf mutant *det2* we have clarified a biosynthetic pathway of campestanol from campesterol *via* (24*R*)-ergost-4-en-3β-ol, (24*R*)-ergost-4-en-3-one and (24*R*)-5α-ergostan-3-one.[3,6] These results suggest an alternative biosynthetic pathway generating active brassinosteroids *via* **2**: campesterol→**1**→(22*S*,24*R*)-ergost-4-ene-3β,22-diol **5**→(22*S*,24*R*)-22-hydroxyergost-4-en-3-one **6**→(22*S*,24*R*)-22-

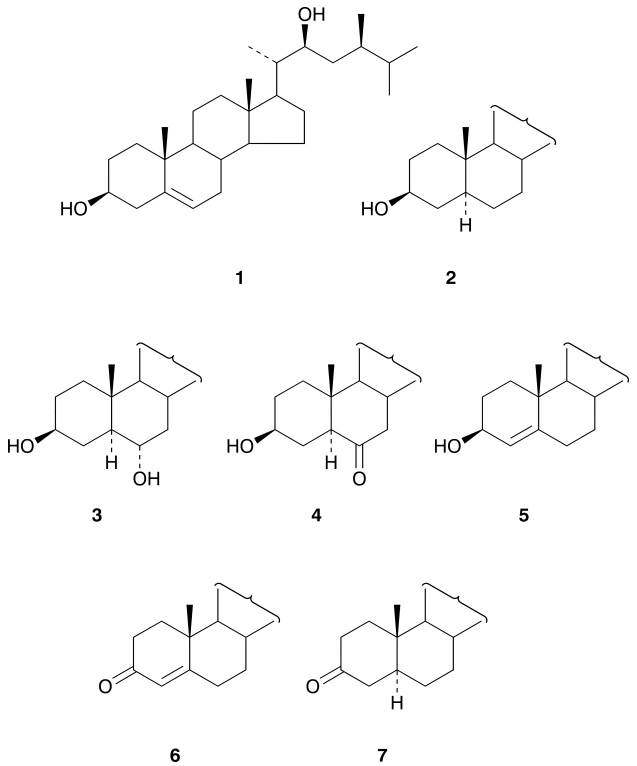
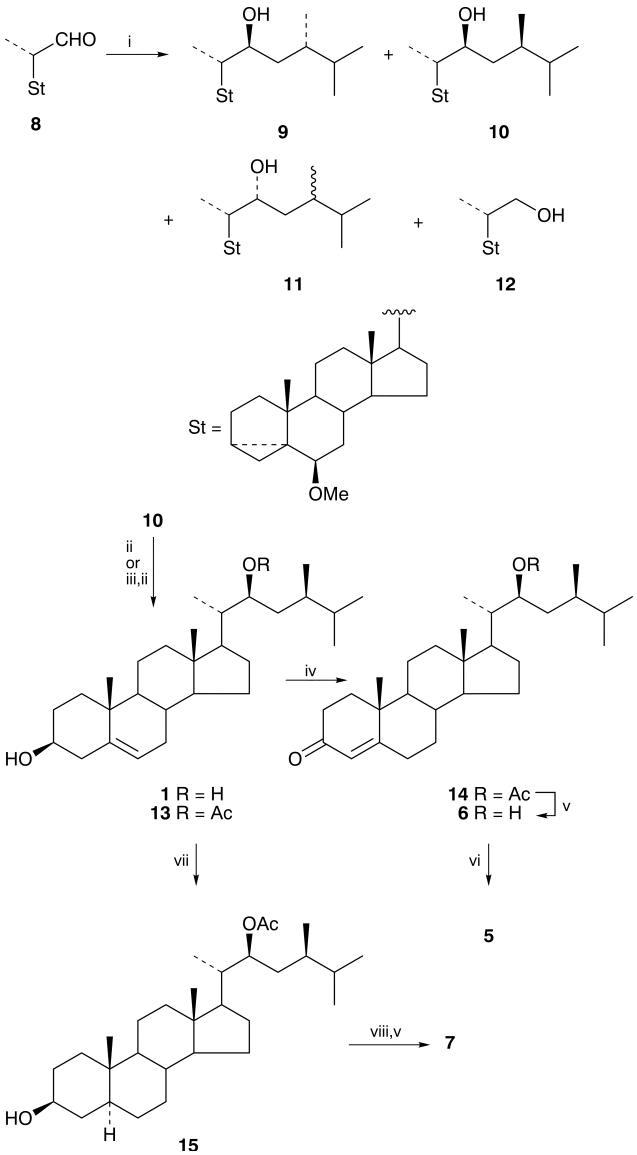



**Fig. 1** Structures of (22*S*)-22-hydroxycampesterol and its related compounds




**Scheme 1** *Reagents and conditions*: i, 2,3-dimethylbutylmagnesium bromide, THF, room temp., 1 h; ii, *p*-TsOH, 1,4-dioxane–$H_2O$, 110 °C, 5 h; iii, $Ac_2O$, pyridine, room temp., overnight; iv, 1-methyl-4-piperidone, $Al(Pr^iO)_3$, toluene, 120 °C, 1 h; v, 5% KOH–MeOH, 1,4-dioxane, 70–80 °C, 1 h; vi, $NaBH_4$–$CeCl_3 \cdot 7H_2O$, THF–MeOH, room temp., 20 min; vii, $H_2$, 10% Pd–C, EtOAc–EtOH, room temp., overnight; viii, Jones reagent, acetone, room temp., 20 min

*To receive any correspondence (*e-mail:* takatuto@juen.ac.jp).

hydroxy-5α-ergostan-3-one **7**→**2**→6-deoxoteasterone and/or **3**→**4**, and then 6-deoxoteasterone and **4** fall into the early and late C-6 oxidation pathways[4a] of the brassinolide biosynthesis, respectively. In order to identify the new possible biosynthetic intermediates **5**–**7** from plants, we needed authentic samples of **5**–**7**. In this paper, we report a convenient synthesis of **1** and its related steroids **5**–**7**.

Our previous synthesis of **1**–**4** has drawbacks of long reaction steps, low selectivity and, hence, low overall yields.[2] Thus, we have now investigated a more convenient and one-step method to construct the desired 22*S*-hydroxy-24*R*-methyl side-chain part by Grignard reaction of a known 22-aldehyde **8**.[7] Cheng *et al.* have previously synthesized **1** as an inseparable mixture of its 24-epimers by the same methodology.[9]

Reaction of **8** with 2,3-dimethylbutylmagnesium bromide provided, after repeated chromatographic separations, the (22*S*,24*S*)-22-hydroxy compound **9** (19%) and its (22*S*,24*R*)-isomer **10** (46%), along with a mixture of (22*R*,24*R*)- and (22*R*,24*S*)-isomers **11** (7%) and a reduced 22-alcohol **12** (23%) (Scheme 1). As expected, the (22*S*)-hydroxy compounds **9** and **10** were obtained as major products. Interestingly, we have found that, with respect to the configuration at the C-24 position, the desired (24*R*)-isomer **10** was obtained as a major product, notwithstanding the use of achiral 2,3-dimethylbutyl bromide. The isolated (22*S*,24*R*)-isomer **10** was converted into the known **1**.[2] Transformation of **1** into **2**–**4** is known,[2] and thus their alternative and convenient synthesis was achieved formally.

Having pure **10** in hand, we have now synthesized the new targets **5**–**7** as follows. Acetylation of **10** and regeneration of a 5-en-3β-ol system gave **13**. Oppenauer oxidation of **13** followed by saponification gave **6**, which was further reduced to provide **5**. Hydrogenation of **13** gave **15**, which was then oxidized and deprotected to afford **7**.

Identification of these possible intermediates **5**–**7** in plant sources and their biological evaluation are now in progress.

Techniques used: $^1$H NMR, EI-MS, EI-HR-MS, GC–MS

References: 9

Figure: 1

Scheme: 1

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